8.9 CANNABINOID PROCEDURE (VIA LC/MS/MS)

8.9.1 Purpose

To qualitatively and/or quantitatively identify the following cannabinoids, Δ^{9}-tetrahydrocannabinol (Δ^{9}-THC), 11-hydroxy-Δ^{9}-tetrahydrocannabinol (THC-OH), and 11-nor-9-carboxy-Δ^{9}-tetrahydrocannabinol (THC-COOH), in submitted evidence using liquid/liquid extraction followed by instrumental analysis with liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS).

8.9.2 Specimen Requirements

Samples for this analysis shall be presumptively positive using the current screening method. This analysis is suitable for whole blood. For additional sample types, see 8.8 THC-COOH.

8.9.3 Apparatus and Equipment

Disposable 15 mL culture tubes and screw caps
Disposable 10 mL culture tubes
Volumetric pipettes and disposable tips
Assorted volumetric glassware
Disposable transfer pipettes
Sample mixer
Centrifuge
Evaporation station
11 mm autosampler vials, inserts, and caps
11 mm crimper
LC/MS/MS, Analyst and/or Cliquid software, compatible computer, and printer

8.9.4 Reagents and Standards

(-)-Δ^{9}-THC certified reference standard
(±)-11-Hydroxy-Δ^{9}-THC certified reference standard
(-)-11-nor-9-Carboxy-Δ^{9}-THC certified reference standard
(-)-Δ^{9}-THC-D_3 certified reference standard (internal standard)
(±)-11-Hydroxy-Δ^{9}-THC-D_3 certified reference standard (internal standard)
(±)-11-nor-9-Carboxy-Δ^{9}-THC-D_3 certified reference standard (internal standard)
Water (H_2O)
Methanol (CH_3OH)
Methanol/Water 1:1
10% Glacial Acetic Acid (CH_3COOH)
Hexane/Ethyl Acetate 9:1
1 M Ammonium Formate (NH_4HCO_2)
Formic Acid (HCO_2H)
Mobile Phase A (99.6% water, 0.2% 1 M ammonium formate, 0.2% formic acid)

Mobile Phase B (97.6% HPLC grade or equivalent methanol, 2% water, 0.2% 1 M ammonium formate, 0.2% formic acid)
Negative potassium oxalate/sodium fluoride fortified bovine whole blood
Note: Bovine blood expires 6 months from date received.

8.9.5 Standard and Reagent Preparation

The following are examples of how to prepare the standards and reagents used in this procedure.

8.9.5.1 Standards

Stock Reference Standard Solution (1)
Δ^9-THC & THC-OH [100 ng/mL] & THC-COOH [500 ng/mL]
Pipette 10 µL of Δ^9-THC & THC-OH [1 mg/mL] certified reference standard and 50 µL of THC-COOH [1 mg/mL] certified reference standard and dilute to 100 mL with methanol.

Stock Reference Standard Solution (2)
Δ^9-THC & THC-OH [1,000 ng/mL] & THC-COOH [5,000 ng/mL]
Pipette 100 µL of Δ^9-THC & THC-OH [1 mg/mL] certified reference standard and 500 µL of THC-COOH [1 mg/mL] certified reference standard and dilute to 100 mL with methanol.

Stock Reference Standard Control Solution
Δ^9-THC & THC-OH [1,000 ng/mL] & THC-COOH [5,000 ng/mL]
Pipette 100 µL of Δ^9-THC & THC-OH [1 mg/mL] certified reference standard and 500 µL of THC-COOH [1 mg/mL] certified reference standard and dilute to 100 mL with methanol.

Reference Standard Solution (Internal Standard)
Δ^9-THC-D_3 & THC-OH-D_3 [1,000 ng/mL] & THC-COOH-D_3 [5,000 ng/mL]
Pipette 100 µL of Δ^9-THC-D_3 [1 mg/mL] certified reference standard, 1,000 µL of THC-OH-D_3 [100 µg/mL] certified reference standard, and 500 µL of THC-COOH-D_3 [1 mg/mL] certified reference standard and dilute to 100 mL with methanol.

Working Reference Calibrator/Control Standard Solutions
To make the working reference calibrator/control standard solutions, add the following amounts to a final volume of 1 mL with potassium oxalate/sodium fluoride fortified bovine whole blood.

CONCENTRATIONS (Δ^9-THC/THC-OH & THC-COOH)	AMOUNT USED	STANDARD SOLUTIONS
1 ng/mL & 5 ng/mL	10 µL	Stock Reference Standard Solution 1
2 ng/mL & 10 ng/mL	20 µL	Stock Reference Standard Solution 1
4 ng/mL & 20 ng/mL	40 µL	Stock Reference Standard Solution 1
10 ng/mL & 50 ng/mL	10 µL	Stock Reference Standard Solution 2 **AND** Stock Reference Control Standard Solution
40 ng/mL & 200 ng/mL	40 µL	Stock Reference Standard Solution 2

8.9.5.2 Prepared Reagents

10% Glacial Acetic Acid Solution
Add 100 mL glacial acetic acid to H_2O and dilute to 1000 mL with H_2O.

Hexane/Ethyl Acetate 9:1
Combine 1800 mL hexane and 200 mL ethyl acetate.

1 M Ammonium Formate
Dissolve 63 g of ammonium formate and dilute to 1000 mL with H_2O.

Mobile Phase A
Add 2 mL 1 M ammonium formate and 2 mL formic acid to H_2O and dilute to 1000 mL with H_2O.

Mobile Phase B
Add 2 mL 1 M ammonium formate and 2 mL formic acid to 20 mL H_2O and dilute to 1000 mL with methanol.

Mobile Phase A: Mobile Phase B 40:60 (Reconstitution Solution)
Add 400 mL of Mobile Phase A to a volumetric flask and dilute to 1000 mL with Mobile Phase B.

Methanol/Water 1:1 (Needle Rinse)
Add 500 mL of methanol to a volumetric flask and dilute to 1000 mL with H_2O.

8.9.6 Procedure

1. Allow all reference standards and case samples to equilibrate to room temperature before beginning procedure.
2. Label, check, and load/unload all samples in accordance with the "Sample Pipetting Check List" (see Appendix section).
3. Prepare working reference calibrator and/or control standards from the stock reference standard solutions.
4. Pipette 1 mL of corresponding case sample, calibrator, positive control, or negative control into the appropriately labeled 15 mL culture tube.
   Note: Smaller sample volumes may be analyzed on a case-by-case basis.
5. Pipette 20 µL of internal standard into each sample to make a final concentration of 20 ng/mL (Δ^9-THC-D_3 & THC-OH-D_3) and 100 ng/mL (THC-COOH-D_3).
6. Add 2 mL of H_2O and vortex briefly.
7. Add 800 µL of 10% glacial acetic acid and mix sample approximately 10 seconds.
8. Add 8 mL of 9:1 hexane/ethyl acetate, rotate (approximately 30 minutes), and centrifuge until separated (approximately 20 minutes).
9. Transfer the upper layer (organic) to the appropriately labeled 10 mL culture tube.
10. Evaporate to dryness with heat (optional) and a dry gas (e.g. nitrogen) in an evaporation station.
    Note: In order to prevent sample evaporation, allow sample to cool before proceeding.
11. Reconstitute the residue with 100 µL of Mobile Phase A/Mobile Phase B 40:60, mix, and centrifuge until separated (approximately 15 minutes).
    Note: Over centrifugation can lead to a reduced sample volume.
12. Transfer to an 11 mm autosampler vial with insert, attempting to avoid transfer any of the particulate matter in the bottom of the tube, and seal with cap.
13. Analyze the samples by LC/MS/MS.

8.9.7 Reporting

Results can be reported if the following criteria are met:

8.9.7.1 Qualitative

8.9.7.1.1 Retention times of drugs identified and internal standards must fall within ± 2% of calibrator or control standards.
Note: Some drug retention times are concentration dependent and a comparison of ± 2% to a calibrator or control standard of similar concentration, used in the calibration curve, shall be acceptable.

8.9.7.1.2 Multiple reactions monitoring (MRM) ion ratios must fall within ± 20% of the calibrators or control standard. If a calibration point is removed then the ion ratio range shall be recalculated from the calibrators used to establish the curve.

Note: Some drug MRM ion ratios are concentration dependent and a comparison of ± 20% to a calibrator used in the curve or control standard of similar concentration shall be acceptable.

8.9.7.1.3 If the control standard concentration is outside the expected value, the drug may be reported as "positive" if the retention time criteria and ion ratio criteria are met.

8.9.7.1.4 Drug concentrations in casework may be reported as "positive" if the drug response ratio (i.e., area of drug/area of internal standard) is equal to or greater than the drug response ratio of the lowest calibrator used to establish the calibration curve.

8.9.7.2 Quantitative

8.9.7.2.1 All of the qualitative result criteria above must be met.

8.9.7.2.2 Sample drug concentrations greater than the highest calibration point, where the results are necessary for interpretation in the case, shall be reanalyzed using smaller sample amounts (e.g., aliquots of 500 μL diluted with 500 μL bovine blood).

8.9.7.2.3 Sample drug concentrations greater than the highest calibration point where the results are not necessary for interpretation in the case may be reported as "greater than…." the highest calibration point.
Note: Drug concentrations of 200 ng/mL for Δ^9-THC/THC-OH or 600 ng/mL for THC-COOH produced no carryover using this procedure.

8.9.7.3 Results

8.9.7.3.1 Any qualitative or quantitative report data not used in a case shall either be lined through and initialed or all the data used shall be highlighted.

8.9.7.3.2 Sample drug concentrations below:

- 1 ng/mL for Δ^9-THC (the lowest calibration point) shall be reported as "No Δ^9-tetrahydrocannabinol detected".
- 1 ng/mL THC-OH (the lowest calibration point) shall be reported as "No 11-hydroxy-Δ^9-tetrahydrocannabinol detected".
- 5 ng/mL for THC-COOH (the lowest calibration point) shall be reported as "No 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol detected".

8.9.7.3.3 Qualitative results shall be expressed as "positive" and include any clarifying remarks, if applicable.

8.9.7.3.4 Quantitative results shall be reported in ng/mL and truncated to the whole number.

8.9.7.3.5 When a definitive conclusion cannot be made, the reason shall be documented on the report (e.g., “insufficient sample for analysis”, “sample unsuitable for analysis”, “results are inconclusive due to sample condition”, etc.).

8.9.8 References

See method validation for extensive bibliography.